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LIVER FUNCTIONS DERANGEMENT AMONG SUBSTANCES ABUSERS

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ABSTRACT

Background: Substance abuse is a worldwide problem with rapidly expanding prevalence. Liver is highly vulnerable to xenobiotics toxicities.

Methods: We have investigated the effect of substances of 5 commonly abused in Egypt (tramadol, opiates, cannabis, barbiturates and benzodiazepines) on liver functions in persons attending to Mansoura university hospitals. After exclusion of cases with chronic liver diseases, participants were screened by enzyme multiplied immunoassay. Positive cases were confirmed by gas chromatography-mass spectroscopy and examined by abdominal ultrsonography and live functions tests were done.

Results: GC-MS showed only 125 positive cases. No abnormalities were detected by ultrasonography in the examined populations. SGOT, SGPT and direct bilirubin were significantly increased, while albumin was significantly decreased in abusers in comparison to nonabusers. Tramadol and other opioids were shown to be the most hepatotoixc agents. In addition, SGOT was shown to be significantly increased to less extent in tramadol and cannabis co-abusers. Furthermore, benzodiazepines were shown to significantly increase serum direct and total bilirubin.

Conclusions: We can conclude that tramadol and other opioids significantly alter liver synthetic and excretory function in parallel to significant increase in liver enzymes among abusers. Moreover, cholestatic hepatitis was observed among benzodiazepine abusers.

Key Words: Hepatotoxicity, Substance abuse, Tramadol abuse, Liver functions

INTRODUCTION

A substance use disorder is a disorder in which the use of one or more substances leads to a clinically significant impairment or distress (1). Substance in this definition is limited to psychoactive drugs. Psychoactive substances are used by humans for a number of different purposes. They include anxiolytics as benzodiazepine, euphoriants as Ecstasy, stimulants as cocaine and nicotine, depressants as sedatives, hypnotics, alcohols and narcotics and hallucinogens as: psilocybin and LSD (2).

Recently, the number of substances abusers appears to have increased. By 1997, 25% of the population reported abuse using illicit drugs at some point in their lives, and 10% within the last years (3). There are numerous medical consequences to recreational drug use. Thus, physicians should consider substance abuse mainly as unexplained illness (4).

Liver is commonly affected by drugs and other toxicants as its main blood supply is coming through the portal vein, which drains the alimentary tract, and carrying the absorbed xenobiotics to the liver (5). Macrophages (Kupffer cells) can be immunologically activated by xenobiotics, leading to generation of oxygen free radicals and may also participate in the production of autoimmune injury to hepatocytes (6) In addition to the liver excretory functions, it is also meta-

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bolically active organs which may give rise more toxic compounds than the parent substances. All these factors increase the vulnerability of liver to toxicities effects of drugs and toxicants (7).

So, the aim of the present study was to evaluate liver functions laboratory tests GOT, SGPT, albumin and bilirubin) in addition to abdominal ultrasound among persons, abusing illicit drugs, attending to Mansoura University Hospitals.

Subjects and methods

The study was conducted in Mansoura University hospitals, Dakahlyia, Egypt between January 2013 and November 2013. This facility is draining large area of the Egyptian Delta.

Study population

The study was designed and conducted after approval from Mansoura Faculty of Medicine research ethical committee. Signed informed consents were taken from all persons involved in the study as controls or substance abusers. Confidentiality of data was respected.

One hundred and eighty persons attended to Mansoura university hospitals as patients, patients relatives or friends were included in the study. Abusers were diagnosed as abusers according to International Classification of Diseases-10 (ICD-10). For all participants, history was taken regarding age, residency, educational level and occupation. Abdominal ulutrasonography examination was performed for all persons involved in the study to evaluate the condition of liver, spleen and presence or absence for ascites. Persons with history of chronic liver diseases or with positive screen for hepatitis C and B viruses were excluded from the study. No stipend was provided, however participants were informed about the results of their livers functions laboratory tests and their viral hepatitis screening.

For drug abuse screening, after having informed consent, 20 ml urine was obtained from each participant and prior to giving any treatment. Each sample was collected in a dry, labeled container. Samples were screened for 5 substances of abuse using enzyme multiplied immunoassay technique (EMIT) Emit® d.a.u. TM (drug of abuse in urine) for opiate, cannabis, benzodiazepines, barbiturates and tramadol. After the initial screens, positive cases were confirmed by gas chromatography-mass spectroscopy (GC-MS) (Hewlett Packard, 6890 series). Then liver functions tests for liver cell injuries (SGOT and SGPT) synthetic functions (albumin) and excretory functions (direct and total bilirubin) were performed for all participants.

Statistical analysis:

All statistical procedures were performed using PRISM 5 (GraphPad Software Inc., San Diego, CA). As data showed non parametric distributions, Mann-Whitney was used for comparison between 2 groups, while Kruskall Wallis and multiple comparisons Dunn's post-tests were used. Fisher exact test and chi-square tests were used to study associations. In the figures, the statistical significance is defined as $P<0.05$. In the figures, Significance is indicated as *** for $p<0.001$,**for $p<0.01$ and *for $p<0.05$.

RESULTS

Demographic data of the studied cases and prevalence of substance abuse:

This study was conducted to evaluate liver functions among substances abusers among attendants to Mansoura university hospitals. 180 attendants (159 males 21 females) have satisfied the inclusion criteria of the study and agave signed consent for participation. Age of participants ranged between 18-50 years with a mean age 29.4±7. Regarding occupations, 45 participants (25%) were manual workers, 28 (15.5%) participants were university students, 35 participants (19.4%) were working as farmers, 44 (24.4%) were working in governmental jobs, while 28 (15.5%) participants have reported no occupations. Regarding residency, 68 (37.8%) participants reported that they live in rural area, while the remaining participants were living in urban area (Table 1).

Our EMIT screen data and GC-MS is shown in figure (1). Only 139 cases showed positive urine samples by EMIT. Positive cases were further confirmed by GC-MS. GC-MS showed only 125 positive cases, from which 27 cases have showed positive confirmed positive samples for more than one substance of abuse (mostly tramadol with cannabis). According to GC-MS data considering single substance abuse, only 39 cases were positive only for tramadol, 21 cases were positive for cannabinoids, 16 cases were positive for opiates, 12 cases were positive for benzodiazepine and 10 cases were positive only for barbiturates.

Abdominal Ultrasonography:

Abdominal ultrasonography showed that both liver and spleen were normal in the studied cases without any focal lesions. Both liver and spleen showed normal homogenous parenchyma without any focal lesions. Also no ascites was detected among the studied population.

Effect of substances of abuse on liver function tests:

Effect of substances of abuse on liver enzymes:

Liver functions were studied in abusers and non-abuser groups. Regarding liver enzymes, SGOT levels among non abusers controls were 23.2±8.6 U/L (mean± Standard deviation). While among abusers SGOT values were 32±12.6

U/L. Mann-Whitney test showed statistically significant difference between both groups with p-vale<0.0001. Thirty-one abusers showed SGOT levels blow the mean of the controls while 59 persons showed less than one fold increase over the nonabusers mean and only 35 cases showed more than one fold increase above the non abusers SGOT levels mean.

Regarding SGPT, our results showed that SGPT level among non abusers was (31±9.5) U/L, while its level among abusers was 40.6±15.3 U/L. There was a significant difference between both groups by Mann-Whitney test (p-value<0.0001). Twenty-six abusers showed SGOT levels less than the mean of the nonabusers while 71 persons showed less than one fold increase over the nonabusers mean and only 28 cases showed more than one fold increase above the non abusers SGOT levels mean. Figure (2) shows the effect of substances of abuse on liver enzymes SGOT and SGPT.

Effect of substances of abuse on serum albumin:

Regarding liver synthetic function test, the nonabusers albumin level was 4.3±0.4 g/dl while the abusers showed albumin level of3.8±0.8 g/dl. Mann-Whitney test showed significant difference between both groups (p-value<0.0001).

The effect of psychoactive substances on albumin levels is shown in figure (3).

Effect of substances of abuse on serum bilirubin:

Regarding effect of abuse on liver excretory functions, direct bilirubin data showed statistically significant differences between the abusers and nonabusers groups (p-value<0.0001; Mann-Whitney). The direct bilirubin values among nonabusers and abusers were 0.3±0.03 mg/dl and 0.5±0.3 mg/dl respectively. However, total bilirubin showed statistically significant difference between both abusers and non abusers (p-value<0.0001; Mann-Whitney). The nonabusers showed total bilirubin values range 0.6±0.15 mg/dl. While the abusers showed total bilirubin levels 1±0.3 mg/dl. Total and direct bilirubin levels among different psychoactive substance abusers are shown in figure (4).

DISCUSSION

This work was conducted to assess the effect of substance abuse on liver. Firstly we have assessed the prevalence of substance abuse in the tested groups using preliminary EMIT assays. Then the positive cases were confirmed by GC-MS. Our results revealed that the mean age of the abusers was 22.5±3.5 years. More than half of these patients (56.41%) were below 30 years. These findings support previous studies which stated that substance abuse is more prevalent in young people (8,9).

Also we have found that drug abuse is more prevalent among males (>95% of abusers). This finding is in agreement with studies by Rodham et al. (2005) (10), Bloor (2006) (11) and Fergusson et al. (2008) (12). However, we must state that the limited number of females in the present study was due to some cultural, social and traditional concepts, so most of them refused to participate in our study.

Interestingly, our study also reported higher prevalence of abuse among manual workers (69% of studied manual workers were abusers). This is may be due to false concepts that these substances will increase their efforts and tolerability to prolonged works. Also abuse was more prevalent in urban areas with less social communications among family members, which may work as a barrier to keep young family members from abuse in the rural areas. Furthermore the study showed more prevalence of abuse among non-educated and high school graduates in comparison to the universities graduates and students.

We have confirmed EMIT data regarding abuse with GC-MS as immunoassay technologies lack specificity and reported to show false-positive and false negative test results **(13).** Therefore, all EMIT positive samples were passed to GC-MS assays for confirmed identification of drugs of abuse and more data robustness.

Also our study showed that tramadol was the commonest drug used among abusers, even exceeding cannabis which was considered in a previous study as the commonest drug of abuse (**14**). That is probably due to its wide spread medical use as analgesic. Regarding the effect of substances of abuse on liver functions, Tramadol was found to significantly increase SGOT and SGPT among its abusers in comparison to controls. Serum aminotransferase levels can be elevated in a small proportion of patients receiving tramadol, particularly with high doses. International and accidental overdoses of tramadol can cause respiratory arrest as well as acute liver failure (**15**). The liver injuries with tramadol may be caused by shock, hypoxia or ischemia secondary to the respiratory arrest. Liver injury attributed to tramadol overdose has also been associated with hyperammonemia, lactic acidosis and hepatic steatosis, suggesting direct mitochondrial injury (***16***). Abusers may attain serum levels higher than the toxic levels especially with persons who are addicts for long durations and are tolerant to tramadol with need of increasing the dose continuously to maintain the first experienced pleasure effect of relief.

Regarding other opiates, our data revealed that opiates inhibited liver functions. This in accordance with the previous findings, Based on 40 autopsies of intravenous heroin abusers, Ilic et al. (2005) (17) had reported that heroin abuse induces significant morphologic changes in the liver tissue (vesicular changes, fatty changes, chronic hepatitis and cirrhosis). Interestingly, these changes severity was found to in-

crease with more prolonged abuse durations. That is mostly due to affection of detoxification liver function with reduced rates of heroin biotransformation. It is also suggested that opioids are not directly toxic to the liver, but cause ischemic liver injury due to respiratory failure causing acute hepatic necrosis and liver failure (18).

In the current study, Also tramadol and other opiates were shown to reduce albumin significantly. This may be related to tramadol effect on the synthetic functions of the liver. However, c this decrease in serum albumin may be due to decreased intake as actually abusers are malnourished, in majority. Also in our study tramadol abusers with hypoalbuminaemia had reported longer periods of abuse (>18 months in 61% of cases), but we did not depend on their history during our data analysis as it is known, from our experience, that abusers, mostly, do not tell the trust. Also we did not investigate albumin in urine in these cases, so we cannot exclude renal origin of the observed hypoalbuminemia.

furthermore, tramadol and other opioids were shown to increase total bilirubin with significantly higher levels of direct bilirubin. This means that the problem is mainly cholestatic hyperbilirubinemia with normal liver conjugation. Cholestasis can be explained with opioids as cholestasis was suggested to be associated with altered state of opioid neurotransmission through a number of evidences (19). Bergasa et al. (1998) (20) reported an opiate withdrawal like reaction in patients with cholestasis due to administration of opiate antagonists. Also cholestatsis was found to be associated with higher blood levels of opioid peptides (21). In addition, Opioid receptors were found to be down regulated in rats with cholestasis (22). All these evidences support the ability of opioids to induce cholestasis especially among abusers who tolerate higher serum levels of opioids as a stage of dependence.

Regarding cannabis, it was found that cannabis alone did not cause significant changes in our laboratory data in comparison to the nonabuser. Interestingly, our data showed that cannabis in combination with tramadol decreases its harmful effect. This may be due to cannabis action as antioxidant shown in previous studies as Hampson et al. (2000) (23) who showed that cannabis had antioxidant and neuroprotective effect on neuronal cell cultures. Our data not in accordance with Borini et al. (2004) (24) who stated that cannabis increased SGOT and SGPT slightly among hepatic patients, but our study was dealing with healthy individuals abusing cannabis and we excluded any participant with chronic liver disease or with positive viral B or C hepatitis.

A part from, cholestatsis, benzodiazepine abusers in the present work did not show significant changes in comparison to the control non abuser groups regarding liver. This is in agreement with the previous studies showing that liver function rarely can be affected benzodiazepines (25,26). Benzodiazepines-induced cholstatic hepatitis was reported in previous studies. Zimmerman et al. (1999) (27) reported cholestatic hepatitis have due to alprazolam, chlordiazepoxide, diazepam, flurazepam, and triazolam, and hepatocellular injury with clorazepate and clotiazepam. Also Larrey et al. (2013)(28) had reported cholestsis with with alprazolam, chlordiazepoxide, clonazepam, clorazepate, clotiazepam, diazepam, flurazepam, triazolam and more recently bentazepam

Regarding barbiturates, our data did not report significant effect on liver enzymes,. That is in accordance with previous barbiturates literature there is no evidence that the conventional barbiturates can cause liver (29). Also no reports of liver injury associated with commonly abused secobarbital or amobarbital.

The findings of this study are clinically important as they show that the commonly used psychoactive substances can significantly affect liver function among totally healthy persons. This should raise the attention of physician to consider substance abuse as a differential diagnosis for any middle age person with abnormalities in liver function tests in normal periodic health check up. These finding also raise our attention to consider substance abuse in any liver insult, as the abusers will be more vulnerable to severe liver impairment with any other condition targeting the liver as hepatotoxins or hepatotrophic viruses. In addition, substance abuse should be considered as a prognostic factor in any hepatic patient. Also, the high surprising prevalence of abuse in our locality is necessitating more planning to control handling of psychoactive substances in Egypt.

CONCLUSION

This work was conducted to evaluate the effect of the commonly abused drugs in Egypt in liver functions. We have found that substances of abuse significantly alter liver synthetic and excretory function in parallel to significant increase in liver enzymes among abusers specially addicts to tramadol and other opioids. Also, cholestatic hepatitis is suspected among benzodiazepine abusers. So we recommend larger further studies, including other abuse substances, for more robust data about the effect of psychoactive substances on the liver and other body systems.

Conflict of interest:

No conflict of interest

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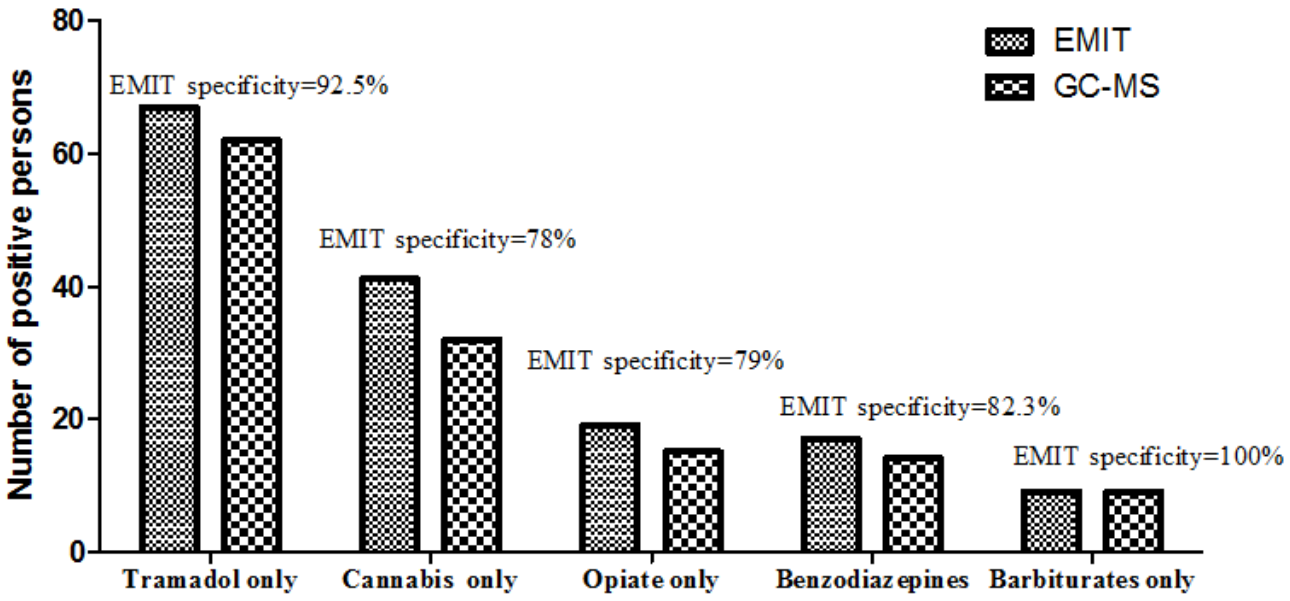

Figure 1: Prevalence of substance abuse in the studied group by EMIT and GC-MS with estimation of EMIT specificity.

Table 1: Demographic characters of participants involved in the study.

Parameter		Non abusers (65)	Abusers (115)	P-value
Age		32.5±5.3	22.5± 3.9	0.002** (a)
Gender	Male	49	110	0.0001*** (b)
	Female	16	5	
Work	Manual workers	14	31	0.002** (c)
	Governmental jobs	28	16	
	Farmers	21	14	
	University students	21	7	
	Not working	17	11	
Residency	Urban	27	85	<0.0001*** (b)
	Rural	38	30	
Education	Universities graduates or students	40	23	0.0112* (c)
	High school graduates	26	37	
	Non-educated	21	33	

(a) means p-value estimated by Mann-Whitney test, (b) means p-value estimated by Fisher exact and (c) means p-value estimated by chi-osqaure

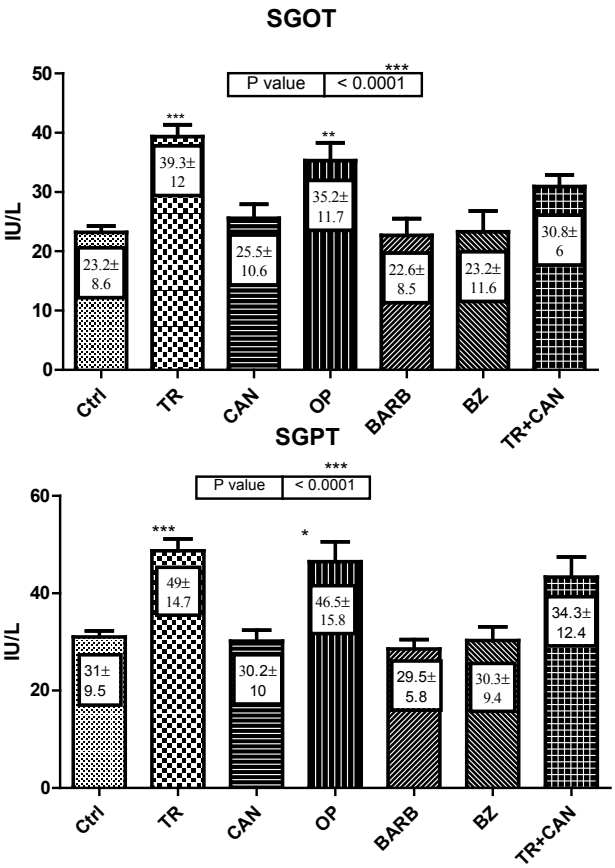

Figure 2: Effect of substances of abuse [tramadol (TR), cannabis (CAN), Opiates (OP), barbiturates (BARB) and benzodiazepines (BZ)] on liver enzymes SGOT and SGPT. Bars are expressing data as means (M)±standard error of means (SEM). Data are shown as means and standard deviation. P-values were calculated by Kruskall Wallis test with Dunn multiple comparison post-test to test the significance of difference from the control. Data is shown as M±SD. (** indicate p-value <0.01, and *** indicate p < 0.001 when compared with vehicle control treated group).

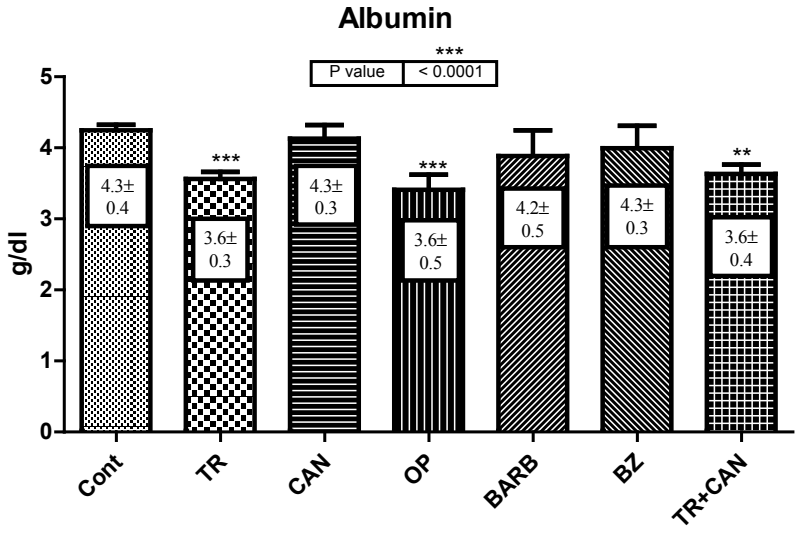

Figure 3: Effect of substances of abuse [tramadol (TR), cannabis (CAN), Opiates (OP), barbiturates (BARB) and benzodiazepines (BZ)] on serum albumin. Bars are expressed as means (M)±standard error of means (SEM). Data are shown as means and standard deviation. P-values were calculated by Kruskall Wallis test with Dunn multiple comparison post-test to test the significance of difference from the control. Data is shown as M±SD. (** indicate p-value <0.01, and *** indicate p < 0.001 when compared with vehicle control treated group).

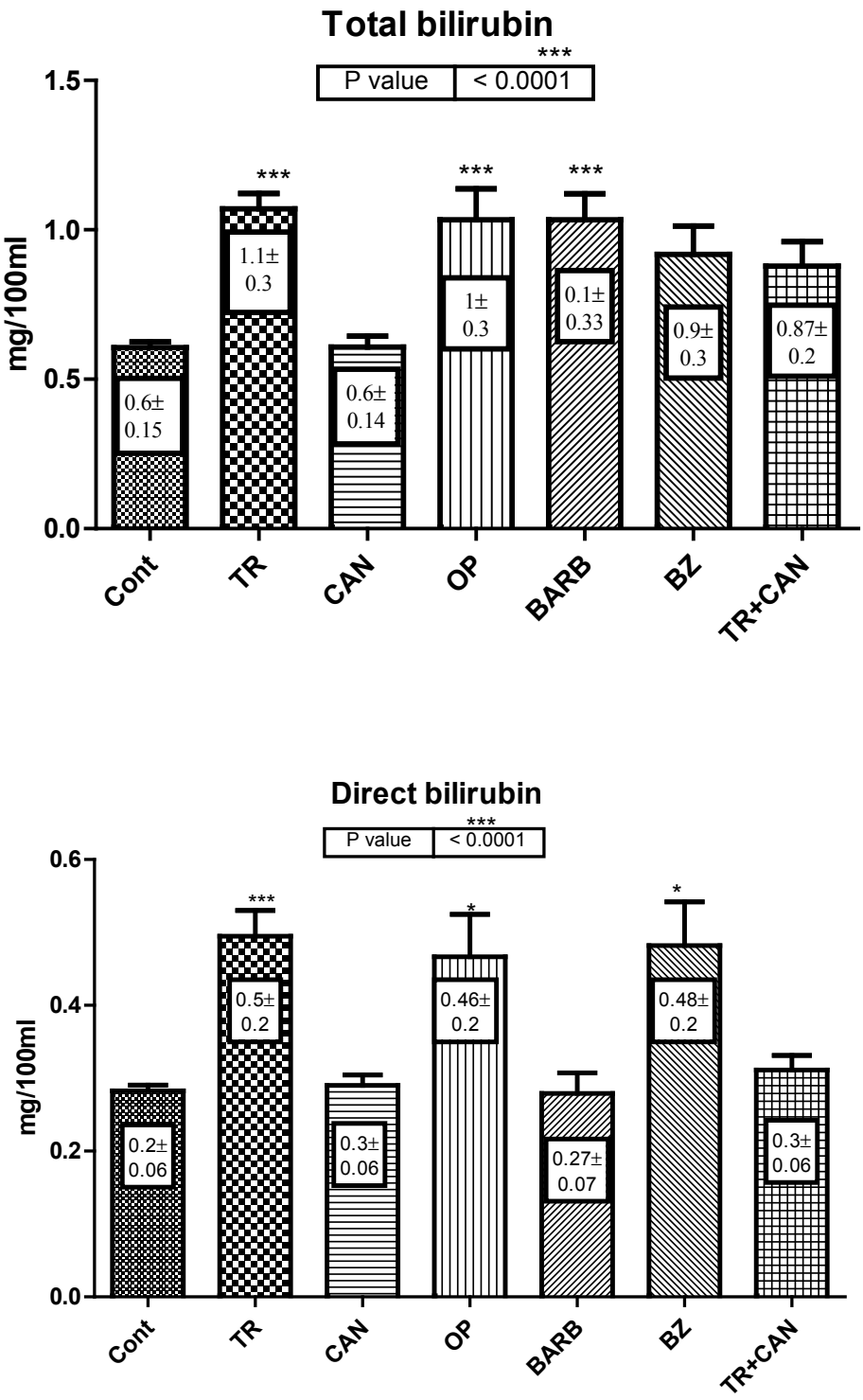

Figure 4: Effect of substances of abuse [tramadol (TR), cannabis (CAN), Opiates (OP), barbiturates (BARB) and benzodiazepines (BZ)] on serum total and direct bilirubin. Bars are expressing data as means (M)±standard error of means (SEM). Data are shown as means and standard deviation. P-values were calculated by Kruskall Wallis test with Dunn multiple comparison post-test to test the significance of difference from the control. Data is shown as M±SD. (* indicate p-value <0.05, and *** indicate p < 0.001 when compared with vehicle control treated group).